RESEARCH ARTICLE

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CHEMOTAXONOMIC STUDY OF SUMATRAN WILD MANGOES (*Mangifera* spp.) BASED ON LIQUID CHROMATOGRAPHY MASS-SPECTROMETRY (LC-MS)

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SUMMARY

The name of an organism is critical to understanding biology. The accuracy of a name is a determinant of the success of the development of herbal medicinal ingredients. Classifying plants based on their chemical constituents may be helpful for taxonomy and discovering new medicinal plants. Metabolite profiling is a new approach in elucidating taxonomy of the *Mangifera* genus as potential medicinal substances. In this study, we evaluated the taxonomic status of wild Sumatran mangoes based on the diversity of chemical compounds. We have classified eight Sumatran wild mango species (*Mangifera magnifica* Kochummen, *M. sumatrana* Miq., *M. laurina* Bl., *M. kemanga* Bl., *M.quadrifida* Jack., *M. foetida* Lour. (type Limus, Batu and Manis), *Mangifera* sp.1 (MBS) and *Mangifera* sp.2 (MH)) based on the similarity of the compounds possessed. The compounds were identified using LCMS and cluster equation analysis using NTSYS. The results suggested that *Mangifera* sp.1 (MBS) and *Mangifera* sp.2 (MH) are not the same. Both should be recommended as a new species. These findings reveal the significance of metabolite content as a taxonomic marker. These results also indicated that *M. sumatrana* and *M. laurina* are not the same species.

Keywords: Chemotaxonomy, LCMS, *Mangifera,* Sumatran wild mangoes

Key findings: These findings reveal the significance of metabolite content as a taxonomic marker. LC-MS method as new evidence in the identification of wild Sumatran mangoes. Based on this metabolite data, two species of wild Sumatran mangoes can be recommended as new species.

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INTRODUCTION

Secondary metabolites are bioactive substances with diverse chemical structures. It's one of the taxonomic evidence for grouping plants (chemotaxonomy). This knowledge provides secondary constituent information for plant classification which may be applied to the species of the genus *Mangifera*, family Anacardiaceae. The chemotaxonomic study of mango stem bark content by Eiadthong *et al.* (2000) is one of them. Recent taxonomic studies using the morphological approach of this genus have been published by Kosterman and Bompard (1993) which succeeded in describing 69 species of *Mangifera,* but problems in grouping closely related species remained. In addition, phylogenetic analysis using DNA of primary constituents have also been reported by some authors (Eiadthong *et al.,* 2000; Yonemori *et al.,* 2002; Hidayat *et al.,* 2012; Suparman *et al.,* 2013; Ariffin *et al.,* 2015; Fitmawati *et al.,* 2016).

Sumatran wild mangoes (*Mangifera* spp.) are unusual varieties because they possess interesting phytochemical properties (Fitmawati *et al.,* 2020). To develop wild mangoes as an herbal medicinal ingredient, it is very important to ascertain. Phytochemical changes occur more quickly than morphological changes. So that the analysis of the relationship of wild Sumatran mango species can be a clue of phytochemical relationship.

Various approaches have been made to determine the position of *Mangifera* members, both morphological and molecular approach. However, the data obtained have not provided a complete solution to the problem. The grouping of certain species still creates ambiguity, such as in determining the position between members of the subgenera *Limus* and *Mangifera*. In addition, several species that do not yet have a specific name (specific epithet) have the potential to be classified as a new species. The accuracy of the name is necessary for the protection and utilization of certain species.

The concept of species in classification can be supported by chemical evidence, such as their antioxidant content. Various species of mangoes are reported to contain high levels of antioxidants. Research by Fitmawati *et al*., (2020) reported that wild Sumatran mangoes (*Mangifera* spp.) contain high levels of antioxidants (gallic acid and quercetin), which are found in *Mangifera* sp.1 (MBS). Pharmacological activity of antioxidant is able to prevent oxidative damage by free radicals (Jain *et al.,* 2019) which has contributed to triggering various chronic and degenerative diseases such as cancer, autoimmune disorders, oxidative, cataracts, rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Wauthoz *et al.,* 2007; Pardo-Andreu *et al.,* 2008; Pham-Huy *et al.,* 2008; Luo *et al.,* 2012; Ronchi *et al.,* 2015).

The pharmacological activity in mangoes is caused by the presence of bioactive compounds. Detection is needed to identify these bioactive components. One study that is effective in revealing metabolic compounds contained in a plant and its potential as new medicinal substances is metabolomic (Shyur and Yang, 2008; Esslinger *et al.,* 2014). Metabolomics is the study that aims to detect as many as possible of the metabolites through a comprehensive analysis of tens or even hundreds of metabolites (Schulze-Kaysers *et al.,* 2015; Nurmaida *et al.,* 2018). Metabolomic analysis in plants can be applied to the development of active secondary metabolites. By conducting investigations and researching the relationship between the compounds in various species of wild mango, it is hoped that a more accurate classification of mangoes species will be developed.

In this study, we examined mangoes from the island of Sumatra including two of them without a specific name, namely *Mangifera* sp.1 (MBS) and

Mangifera sp.2 (MH). Researching the taxonomic status and developing its potential as a medicinal plant needs to be done, so that other taxonomic supporting methods are needed such as chemotaxonomy to identify unknown species, strengthen species status or reconstruct conventional taxonomic groupings. In addition, chemical evolution takes place faster than morphological evolution, so the use of a series of phytochemical data is needed to support accurate classification of the genus *Mangifera.* Phytochemical constituents are important because they are used as markers to classify and build plant relationships (Ganneru *et al.,* 2019). Morphologically, *Mangifera* sp.1 has small adult leaves 10-12 cm, light green in color, and closely packed leaf bones. *Mangifera* sp.2 has a leaf size of about 20-30 cm, the leaf bones are not tight, and the leaves are thick and fleshy (Fitmawati *et al.,* 2013).

Identification of metabolite content or through metabolomic profiling for cultivated mangoes has been widely studied (Tank *et al.,* 2016; White *et al.,* 2016; Hiralben *et al.,* 2018; Pradhan *et al.,* 2018; Ganneru *et al.,* 2019). Conversely, in some wild mangoes from the island of Sumatra, Indonesia has never been done. In this study, a methanol extract of wild mango leaves was used for metabolite profiling using metabolomic analysis with an analytical technique which is LC-MS (Liquid Chromatography-Mass Spectrometry).

This technique has various advantages including being able to detect a wide range of metabolite class variations, revealing new or minor metabolites, having very high sensitivity and specificity (Farag *et al.,* 2012; Khairan *et al.,* 2009). It is hoped that the accuracy of the information on the content of metabolite compounds produced from wild mangoes can be used as a new phytotherapy agent. Therefore, the identification of metabolite compounds through the analysis of metabolomic profiling of eight species of wild mangoes is important to obtain as supporting taxonomic data and a basis for preclinical and clinical testing towards the stage of making pharmaceuticals.

MATERIALS AND METHODS

Equipment and materials

Tools: vacuum rotary evaporator, pH tester, Liquid Chromatography-Mass Spectrometry instrument with detailed LC System (ACQUITY UPLC® H-Class System (waters, USA)); LC Column (ACQUITY UPLC® HSS C18 (1.8 µm 2.1x100 mm) (waters, USA)); and Mass Spectrometer (Xevo G2-S QTof (waters, USA)). Ingredients: 50 g of wild mango leaf powder (*Mangifera magnifica* Kochummen (Collected in Kampar Regency, Riau Province), *M. sumatrana* Miq (Pekanbaru)., *M. laurina* Bl. (Kampar Regency), *M. kemanga* Bl. (Kuantan Singingi Regency), *M. quadrifida* Jack. (Rokan Hulu Regency), *Mangifera* sp.1 (MBS) (Bukit Suligi Protected Forest Rokan Hulu), *Mangifera* sp.2 (MH) (Syarif Hasyim Forest Park, Siak Regency and *M. foetida* Lour. (type Limus, Manis and Batu) (South Sumatera Province) eight species of wild mango in Sumatra with sufficient sample size for research), aquades, acetonitrile, methanol, and dichloromethane.

Plant preparation and extraction

Dried mango leaves were ground into a powder using an herbal grinder (Getra IC-06B). A total of 50 grams of dry simplicia powder of mango leaves were extracted by maceration using 1 liter of methanol soaked for 24 hours and macerated three times. The results of the macerate were filtered and concentrated using a vacuum rotary evaporator at a temperature of 50◦C until a thick extract was obtained. The thick extract is dissolved with water to form a dilute solution. The solution was eluted using the OASIS SPE (Solid-Phase Extraction) method to separate the pure extract from the impurities for analysis.

Identification of metabolic compounds of mango leave extract based on LC-MS

Identification of metabolites in samples of wild mango leaves was carried out by two injections into the LC-MS system (5 µL per injection). Samples were filtered using a 0.2 µm syringe filter, put into vials and injected into the LC-MS system. The resulting data are in the form of a Chromatogram (LC) and Spectra (MS) (Taupik *et al*., 2020).

LC-MS analysis

Chromatogram data were processed using the MassLynx V4.1 SCN884 program ©2012 Waters Inc. software. The analysis stage begins by converting the chromatogram in the form of BPI (Base Peak Intensity). Each peak is analyzed one by one to obtain spectra that will be used to obtain the molecular formula contained in it to match the molecular formula in the Chemspider database (www.chemspider.com). Molecular formulas that match the database will display the names of the compounds from the formula and can be grouped by main group using the PubChem database, KEGGs (Kyoto Encyclopedia of Genes and Genomes) and Human Metabolome Database.

Numerical taxonomic analysis

Determination of variation and similarity among wild mangoes was carried out by checking the presence or absence of 88 compounds detected. This similarity is measured based on the similarity between species which neither produce certain compounds (score 0) and also based on the content of similar compounds possessed by these species (score 1). Observation data in the form of scores were processed to obtain a metabolite similarity matrix using the SIMQUAL (Similarity for Quality Data) procedure. Furthermore, this similarity matrix is used to group SAHN (Sequential Agglomerative Hierarchical and Nested Clustering), the similarity coefficient with the SM (Simple Matching) method and clustering using the UPGMA (Unweighted Pair Group Method Arithmetic Average) method in the NTSYS pc version 2.02 (Numerical Taxonomy and Multivariate System). Principal component analysis (PCA) analysis uses multivariate analysis using Minitab software (version 16.0).

RESULTS

Phytochemical profiles of *Mangifera* spp.

The metabolite compounds detected from eight wild mangoes species were obtained based on the analysis of the chromatogram on the dominant peaks (abundance ≥ 50%) and a total of 88 metabolites were detected (Table 1).

These compounds could be classified into alkaloids, alkanes, phenolics (flavonoids, phenylpropanoids, gallic acids), amino acids, benzene, fatty acids (lipid sterols, fatty acyl), benzoic acid, organic aromatics, diterpenoids, furochromone, carboxylic acids, and acetic acid (Table 1). The main groups obtained are based on main peak analysis to obtain molecular formulas, then matched with the PubChem database, KEGG (Kyoto Encyclopedia of Genes and Genomes) and Human Metabolome Database. However, there are also some metabolite compounds that are difficult to identify for groups and their benefits due to limited information in the database. According to (Borden *et al.,* 2020), generally databases only list groups of complex or large compounds and known compounds, whereas in this study the compounds detected were single compounds that were small in size with a range of 100-700 masses per ion (m/z) with rare or unknown systematic names.

Table 1. Total compounds detected in *Mangifera* spp.

Alkaloids	RT	m/z	sp.	Alkaloids	RT	m/z	sp.
[1]Methyl (2S,3R,4S)-4-{[(1R,4R)-2-acetyl-4,6,7-trihydroxy-1,2,3,4-tetrahydro-1-isoquinolinyl]methyl}-2-(α-D-galactopyranosyloxy)-3-vinyl-3,4-dihydro-2H-pyran-5-carboxylate	7.52	581.56	B, Mn	[15]methyl 9-[(2S,3S,4S,5R)-5-[[(2R,3R,4R)-2-ethyl-3,4-dihydroxy-pyrrolidin-1-yl]methyl]-3,4-dihydroxy-tetrahydrofuran-2-yl]oxynonanoate	7.34	433.53	Sm
[2]9H,16H-Quinazolino[2',3':2,3]pyrimido[6,1-b]quinazoline-9,16-dione	5.01	314.29	B	[16]N-{2-[(11aS)-5-(4-Isopropylphenyl)-1,3-dioxo-5,6,11,11a-tetrahydro-1H-imidazo[1',5':1,6]pyrido[3,4-b]indol-2(3H)-yl]benzoyl}-L-isoleucine	16.29	592.68	
[3]N,N-Diethyl-2-(4-pyridinyl)-4-quinazolinamine	14.26	278.35		[17]Quinoxalino[2',3':3,4]cyclobuta[1,2-b]quinoxaline	7.08	256.26	
[4]Ethyl 4-[2-(4-methylphenyl)-4-oxo-3(4H)-quinazolinyl]benzoate	13.03	303.52	L, Mn	[18]6-O-(2-Acetamido-2-deoxyhexopyranosyl)-3-O-butyl-1,2-O-isopropylidenehexofuranose	7.91	479.51	Mn
[5]2-Hexyl-3,5-Dipentylpyridine	13.03	303.52	H	Alkanes	RT	m/z	sp.
[6]N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-oxo-4-(1-piperidinyl)butanamide	5.69	370.44	Mg	[19]4,7,7-Trimethyl-3-Oxobicyclo(2.2.1)Heptane-1-Carboxylic Acid	6.70	196.24	B,H,K, La, Mg, Q, S, Sm, Mn
[7]1-[(1'-Methyl-1,4'-bipiperidin-4'-yl)methyl]-3-[2-methyl-2-(1-piperidinyl)propyl]urea	17.35	393.61	Mg, S	[20](6R,8S,9R,12S,16S,17R)-6-[(4-Benzoylbenzyl)oxy]-9,12,16,17-tetrahydroxy-8-(2-methyl-2-propanyl)-2,4,14,19-tetraoxahexacyclo[8.7.2.01,11.03,7.07,11.013,17]nonadecane-5,15,18-trione	7.52	636.59	L
[8](11β)-11,17-Dihydroxy-3,20-dioxopregn-4-en-21-yl 4-{[2-(1H-imidazol-5-yl)ethyl]amino}-4-oxobutanoate	4.27	555.66		[21]4-(2,4-Di-tert-pentylphenoxy)butyric acid	13.28	320.46	Q, S
[9]1,5-Anhydro-1-(1-{[(4-butyl-1-{[(5-methyl-2-oxo-1,3-dioxol-4-yl)methoxy]carbonyl}-2-pyrrolidinyl)carbonyl]amino}-2-methylpropyl)-5-propylpentitol	4.27	556.64	Q	[22]4-((2-oxohexadecanoyl)amino)butanoic acid	13.28	355.51	S
[10]1-(4-{4-Methyl-5-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazol-3-yl}-1-piperidinyl)-2-(1H-tetrazol-1-yl)ethanone	5.67	370.41	Q, S	[23](2R,4S,5R,6R)-5-Acetamido-4-hydroxy-2-{[(3aR,5R,5aS,8aS,8bR)-2,2,7,7-tetramethyltetrahydro-3aH-bis[1,3]dioxolo[4,5-b:4',5'-d]pyran-5-yl]methoxy}-6-[(1R,2R)-1,2,3-trihydroxypropyl]tetrahydro-2H-pyran-2 -carboxylic acid	4.65	551.53	Sm
[11]2-Ethylsulfanyl-4,6-diphenyl-nicotinonitrile	13.86	316.41	Q	[24]1-[3-(Carbamoylamino)propyl]-3-(2,4-dimethoxy-3-methylphenyl)-5-(3-nitrophenyl)-4,6-dioxooctahydropyrrolo[3,4-c]pyrrole-1-carboxylic acid	7.77	555.53	
[12](4E)-1-[2-(Diethylamino)ethyl]-4-[hydroxy(4-isobutoxy-3-methylphenyl)methylene]-5-(3-phenoxyphenyl)-2,3-pyrrolidinedione	4.27	556.69	S	Flavonoids (flavones)	RT	m/z	sp.
[13]1-[(1'-Methyl-1,4'-bipiperidin-4'-yl)methyl]-3-[2-methyl-2-(1-piperidinyl)propyl]urea	17.26	393.61		[25]Linaroside	5.01	476.43	B
[14](2R,3R)-2,3-Dihydroxysuccinic acid - (2E)-6-amino-2-imino-4-(1-piperidinyl)-1(2H)-pyrimidinol (1:1)	1.25	360.15	Sm	[26]Morin / Quercetin	4.51	302.23	B, H, K, Mn

Table 1 (cont'd).

Flavonoids	RT	m/z	sp.	Amino acid	RT	m/z	sp.
[27]Quercitrin	4.51	448.37	B, K, Mn	[46]α-Glutamyltyrosylthreonyltryptophan	6.30	597.61	
[28]2,2'-[(2,3,4-Trimethoxyphenyl)methylene]bis(3-hydroxy-6-methyl-4H-pyran-4-one)	6.30	430.40	B, L	[47]Tryptophylphenylalanylalanylthreonine	7.26	523.58	B
[29]Luteolin	6.78	286.23	B,L,Mn	[48]Glycyl-L-α-glutamyl-L-α-glutamyl-L-α-aspartyl-L-aspartic acid	7.26	563.46	
Flavonoids (isoflavones)	RT	m/z	sp.	[49]2-Amino-2-tetradecyl-1,3-propanediol	11.243	287.481	BL
[30]Sophoricoside	7.10	256.25	B,L,Mn	[50]N-Tetracosanoyl-β-alanine	16.07	439.71	B
Flavonoids (flavones)	RT	m/z	sp.	[51]N-[(3-Oxo-3,4-dihydro-1(2H)-quinoxalinyl)carbonyl]-L-isoleucyl-L-asparagine	4.69	419.43	K
[31]liquiritigenin	7.10	256.25	B, L				
Flavonoids	RT	m/z	sp.	[52]Serylthreonylphenylalanyllysin	4.99	481.54	La
[32]Methyl (1S,4aS,5R,7aS)-1-(β-D-glucopyranosyloxy)-7-(hydroxymethyl)-5-{[(2Z)-3-(4-hydroxyphenyl)-2-propenoyl]oxy}-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-4-carboxylate	550.509	550.509	B,L,Mn	[53]Alanylarginylhistidylhistidine	519.55	519.57	Q
[33]Sophoraflavone A	7.26	562.51	B	[54]Asparaginylleucylphenylalanyltryptophan	13.86	578.65	
[34]3'-Sinapoylsweroside/(4aS,5R,6S)-1-Oxo-5-vinyl-4,4a,5,6-tetrahydro-1H,3H-pyrano[3,4-c]pyran-6-yl 3-O-[(2E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-2-propenoyl]-β-D-glucopyranoside	7.52	564.53	B,L,Mn	[55]Phenylalanylglutaminylglycylisoleucine	6.98	463.52	
[35]7-Hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one	8.53	268.26	L	[56]N2-[(2S)-2-(2,4-Dioxo-1,4-dihydro-3(2H)-quinazolinyl)-3-phenylpropanoyl]-L-glutaminyl-L-threonine	7.55	539.53	Sm
[36]Myricetin	5.31	318.23	K				
[37]Hyperoside	5.31	464.37					
Flavonoids (Xanthone)	RT	m/z	sp.	Benzene	RT	m/z	sp.
[38]Scortechinone F	16.29	592.67	K,S	[57]N,4-Bis(1-phenylethyl)-N-[4-(1-phenylethyl)phenyl]aniline	17.19	481.67	Sm
[39]Mangiferin	4.51	422.34	H				
Flavonoids	RT	m/z	sp.	[58](1S,2R,4R,5R,6S,8R,10S,11S,12R,14R,15R,16R,19S,21R)-4,21-Diacetoxy-6-(3-furyl)-12,19-dihydroxy-5,11,15-trimethyl-3-oxo-9,17-dioxahexacyclo[13.3.3.01,14.02,11.05,10.08,10]henicos-16-yl 4-nitrobenzoate	7.26	723.72	B
[40]Gardenin E	4.27	390.34	S				
[41](13R)-13-[(2R,3R,4R,5R)-3,4-Dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]-13-hydroxy-5-oxotridecyl β-D-glucopyranoside	4.27	523.61					
Fenilpropanoids	RT	m/z	sp.	[59]N-Phenyl-N,4-bis(1-phenylethyl)aniline	15.94	377.52	K,Q,Sm
[42]4-Methoxy-2-(2-methyl-2-propanyl)phenol	9.98	180.24	B, H, K,La, Mg,Q,S, Mn	[60]1,1'-[2,2-Propanediylbis(4,1-phenyleneoxy)]bis(2,4-dinitrobenzene)	4.53	560.46	La
[43]4-Allyl-2-Methoxy-3-Methylphenol	6.72	178.22	H,Q,S	[61]N,N'-[Methylenebis(6-hydroxy-3,1-phenylene)]bis(4-nitrobenzamide)	5.37	528.47	
[44]2,3',4,5'-Tetramethoxystilbene	13.86	300.34	Q				
Phenolic (gallic acid)	RT	m/z	sp.	[62]2-{(E)-[6-(3,4-Dimethoxyphenyl)-8-ethoxy-1,3-dimethyl-4H-cyclohepta[c]furan-4-ylidene]amino}aniline	11.51	444.52	S
[45]2,3-Dihydroxy-5-{[2-(2-methoxyethoxy)ethoxy] carbonyl} phenyl 3,4,5-trihydroxybenzoate	1.29	424.35	La				

Table 1 (cont'd).

Lipid sterols (Ester Cholesteryl)	RT	m/z	sp.	Others	RT	m/z	sp.
[63]3β)-Cholest-5-en-3-yl (15,16-dihydroxy-4,7,10,13-tetraoxahexadec-1-yl)carbamate	13.28	694.52	S	[76]Isoxepac	6.30	268.26	B,L
Fatty acyl	RT	m/z	sp.	[77]Ethyl N-(Cyclohexylcarbonyl)Glycinate	6.70	213.27	H,Q,S
[64](1S,2S,7R,16S,18S,20R)-11-Hydroxy-20-(hydroxymethyl)-16-methoxy-6,6,7,20-tetramethyl-10,18-bis(3-methyl-2-buten-1-yl)-3,8,19-trioxahexacyclo[14.4.1.02,14.02,18.04,12.05,9]henicosa-4,9,11,14-te traene-13,17-dione	7.77	579.29	Sm	[78]3R,4S,4aS,9bS)-3-{(Cyclopentylacetyl)[2-(2-methoxyphenyl)ethyl]amino}-8-formyl-4-hydroxy-N-(2-hydroxyethyl)-6-methoxy-3,4,4a,9b-tetrahydrodibenzo[b,d]furan-1-carboxamide	16.29	592.67	H
[65]Eriojaposide A	5.69	502.55	Mg,Q,S	[79]1-(Cyclohexylmethyl)-5'-O-tritylinosine	17.42	606.71	La,Sm
[66]12-Phenyldodecanoic acid	12.57	276.41	Mn	[80](3-Methyl-4-nitrosophenyl)imino]di-2,1-ethanediyl dimethanesulfonate	1.25	380.43	Mg,Q
Benzoic acid	RT	m/z	sp.	[81]Methylpiperazinobenzenediamine	7.461	206.287	
[67](1R,2R)-2-[(4-Hydroxybenzoyl)amino]cyclopentyl 3,5-dihydroxy-4-[2-hydroxy-6-(1H-tetrazol-5-yl)benzoyl]benzoate	5.37	545.50	La	[82]N,N,N-Tris(2-hydroxyethyl)-1-butanaminium hydroxide	7.46	223.31	Mg
[68]2-(Allylamino)-2-oxoethyl 4-{[(2-methyl-5-nitrophenyl)sulfonyl]oxy}benzoate	5.69	434.42	Mg	[83]N-(3-Isopropoxypropyl)-3-[6-(1-pyrrolidinyl)[1,2,4]triazolo[4,3-b]pyridazin-3-yl]propanamide	13.28	360.45	Q
[69]2-Heptyl-5-methylisophthalic acid	13.86	278.34	Q,Sm	[84](2E)-N-{4-[(3-Aminopropyl)amino]butyl}-N'-{6-[(diaminomethylene)amino]hexyl}-2--butenediamide	13.28	383.53	
Organic aromatic	RT	m/z	sp.				
[70]N-Isopropyl-2-methyl-3-phenyl-5-propylpyrazolo[1,5-a]pyrimidin-7-amine	10.21	308.42	L	[85]Propane, 1,3-bis(4,6-diphenyl-1,3,5-triazin-2-yl)	12.75	506.60	
[71]N4-[4-(Diethylamino)-2-methylphenyl]-6-methyl-N2-pentyl-2,4-pyrimidinediamine	13.28	355.52	Q	[86]2-(3,4-Dimethylphenyl)-4-[(2E)-2-{6-oxo-5-[3-(5H-tetrazol-5-yl)phenyl]-2,4-cyclohexadien-1-ylidene}hydrazino]-5-phenyl-2,4-dihydro-3H-pyrazol-3-one	12.75	528.56	S
Diterpenoid	RT	m/z	sp.				
[72]Aphidicolin	13.28	338.48	Q				
Furochromone	RT	m/z	sp.				
[73]khelloside	4.19	408.35	La,Mg, Q,S				
Carboxylic acid	RT	m/z	sp.				
[74](2R,3R,4S,5R)-3,4,5,6-Tetrahydroxy-2-{[(2S,3R,4R,5R)-2,3,4,5,6-pentahydroxyhexyl]amino}hexanoic acid	1.71	359.32	La,Mg, Q	[87]5-[(4-Methyl-2-nitrophenoxy)methyl]-N'-[(Z)-(4-nitrophenyl)methylene]-2-furohydrazide	3.81	425.10	
Acetic acid	RT	m/z	sp.				
[75](2S)-N-{(4R,4aS,6R,8S,8aR)-6-[(2R)-2,3-Dihydroxypropyl]-8-methoxy-7,7-dimethylhexahydropyrano[3,2-d][1,3]dioxin-4-yl}-2-hydroxy-2-[(3S,5R,7R,8S)-5-methoxy-7,8-dimethyl-1,6-dioxaspiro[2.5]oct-5-yl]acet amide	5.69	519.58	Mg, S	[88](5-Methyl-1-phenyl-1H-pyrazol-4-yl){3-[4-(2H-1,2,3-triazol-2-ylmethyl)-1H-1,2,3-triazol-1-yl]-1-azetidinyl}methanone	4.65	389.41	Sm

Note: B (*M. foetida* Lour. (type Batu)), Mn (*M. foetida* Lour. (type Manis)), L (*M. foetida* Lour. (type Limus)), H (*Mangifer* sp.2), Mg (*M. magnifica* Kochummen), S (*Mangifera* sp.1 (MBS)), Q (*M. quadrifida* Jack.), Sm (*M. sumatrana* Miq.), K(*M. kemanga* Bl.), and La (*M. laurina* Bl.).

Table 2. Similarity matrix between eight species of wild mangoes based on on the 88 dominant compounds detected.

Species	B	Mn	L	sp2	Mg	sp1	Q	Sm	K	La
B	1.000									
Mn	0.807	1.000								
L	0.795	0.829	1.000							
Sp2	0.727	0.829	0.772	1.000						
Mg	0.659	0.761	0.727	0.818	1.000					
Sp1	0.568	0.670	0.636	0.772	0.772	1.000				
Q	0.545	0.647	0.613	0.750	0.750	0.704	1.000			
Sm	0.602	0.704	0.693	0.761	0.715	0.625	0.647	1.000		
K	0.738	0.840	0.761	0.875	0.806	0.738	0.715	0.772	1.000	
La	0.681	0.784	0.750	0.840	0.840	0.727	0.727	0.761	0.829	1.000

Note: B (*M. foetida* Lour. (type Batu)), Mn (*M. foetida* Lour. (type Manis)), L (*M. foetida* Lour. (type Limus)), sp2 (*M.* sp2. (MH)), Mg (*M. magnifica* Kochummen), sp1 (*M.* sp1. (MBS)), Q (*M. quadrifida* Jack.), Sm (*M. sumatrana* Miq.), K(*M. kemanga* Bl.), and La (*M. laurina* Bl.).

Similarity coefficient of *Mangifera* spp. based on metabolite compounds

Based on the results of the similarity coefficient matrix, the compound content of all samples measured based on the scoring results has a similarity value range ranging from 0.54 to 0.87 of the total 88 identified dominant compounds (Table 2). The coefficient value (Kf) of 0.54 is the lowest Kf owned by B (*M. foetida* Lour. Batu) and Q (*M. quadrifida* Jack.). The content of compounds which are neither produced by these two species is as much as 46 compounds and the total presence of similar compounds is as much as two compounds (phenylpropanoids and alkanes). The highest Kf is 0.87 owned by H (*Mangifera* sp.2 (MH) and K (*M. kemanga* Bl.). The similarities of the compounds in these two species are 76 compounds with the total presence of the same compound as many as three alkane group compounds, flavonoids (flavones), and phenylpropanoid. This shows that based on the production of these two types of metabolites were very closely related. Similarity matrix between eight species of wild mangoes are summarized in Table 2.

Based on cluster analysis of eight species of *Mangifera* spp. using 88 variations of metabolite compounds, a dendrogram was obtained which formed two main clusters. The first cluster consists of sub-clusters IA (*M. foetida* Lour. (type Batu)), *M. foetida* Lour. (type Manis), *M. foetida* Lour. (type Limus), *M.* sp.2 (MH), *M. kemanga* Bl., *M. magnifica* Kochummen, and *M. laurina* Bl.) and the IB sub-cluster (*M. sumatrana* Miq.) (Figure 1). The second cluster consists of *M.* sp.1 (MBS) and *M. quadrifida* Jack. People would expect that the mangoes will be grouped according to their respective subgenera into the first and second clusters according to the morphological classification. However, the existing sample does not show such a grouping pattern. Wild mangoes cluster based on the equation of the compounds contained or not contained. Therefore, these results need to be reviewed together with the results of the PCA analysis in order to provide a better understanding of the estimated phenetic relationships between the units under study.

Principal component analysis of *Mangifera* spp. based on metabolite compounds

Based on this research, the principal component analysis resulted in 88 factors called main components. Of the 88 main components formed, five main components were selected (PC1, PC2 PC3, PC4, PC5) which were able to explain the cumulative diversity of 74% of the total diversity of 159 characters (Table 3).

Table 3. The Principal Component (PC) values of eight *Mangifera* spp. based on the 88 dominant compounds detected.

Principal Component (PC)	PC1	PC2	PC3	PC4	PC5
Eigenvalue	19,755	15,067	12,607	9,813	7,860
Proportion (%)	22,40	17,10	14,30	11,20	8,90
Cumulative (%)	22,40	39,60	53,90	65,00	74,00
Number of compounds with Eigenvalue >1	40	27	26	45	21

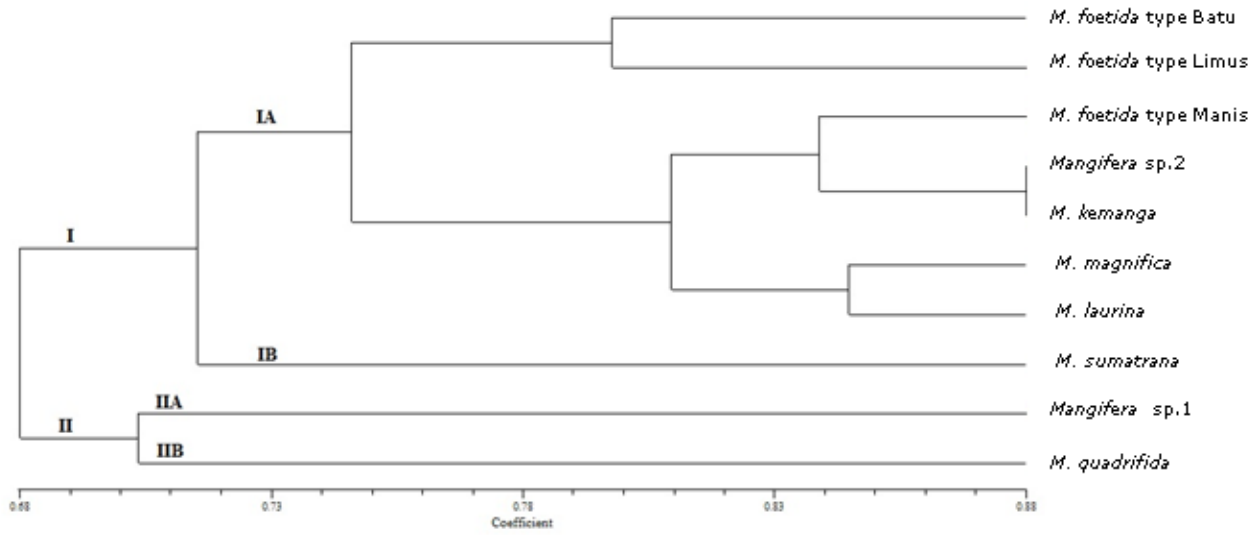

Figure 1. Dendrogram of eight species of *Mangifera* spp.

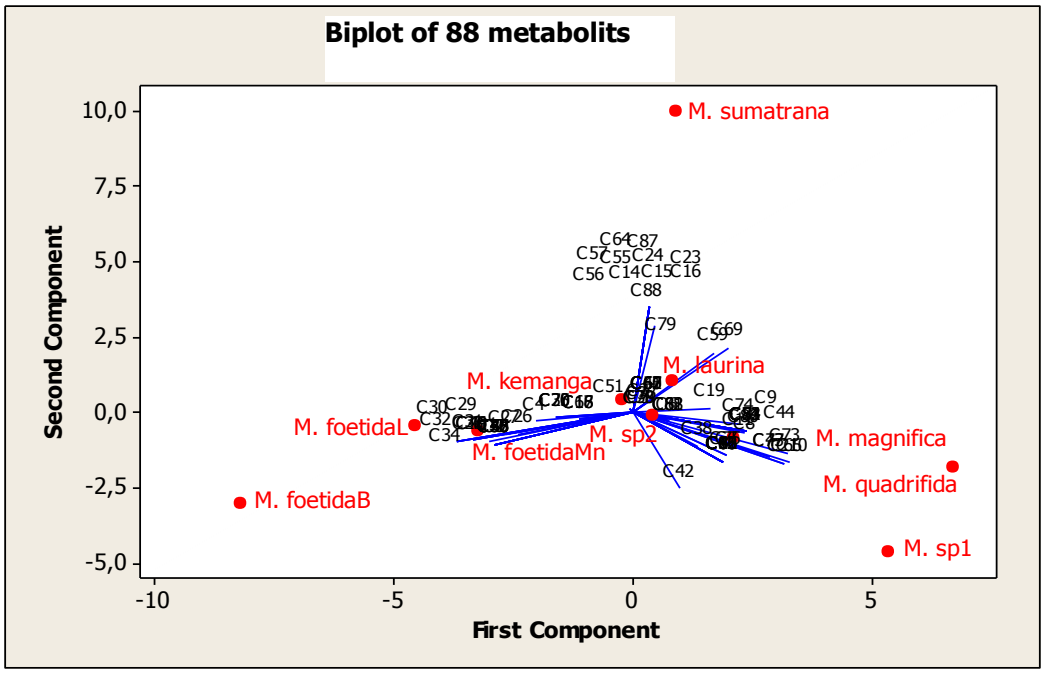

Figure 2. Grouping of eight species of *Mangifera* spp. based on PC1 and PC2 on a biplot.

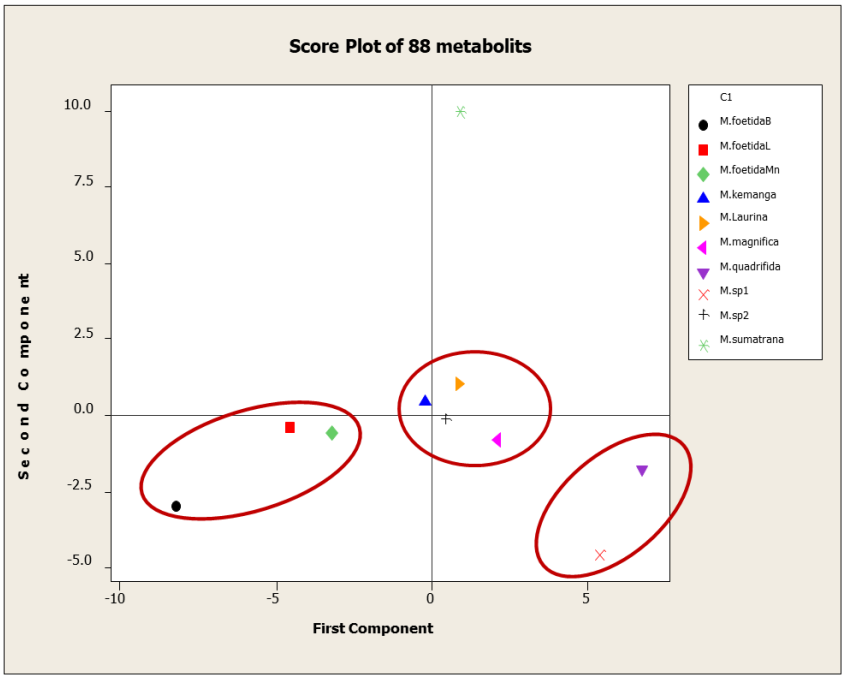

Figure 3. Grouping of eight species of *Mangifera* spp. based on PC1 and PC2 on a score plot.

Sartono *et al.,* (2003) stated that the character with the largest feature vector value is the main character of the component compiler. The five main components are used as the basis for determining the compounds that are incorporated in these components.

The principal components were visualized in Figure 2 and Figure 3. The cumulative diversity of the two principal components (PC1, PC2) is 39.6%. Each of the principal components contributed 22.4% and 17.15% of the variation, respectively. PC1 has a feature vector of 40 metabolite compounds and four compounds (C29, C30, C32, C34) that are most influential in the PC1 grouping (Figure 2). The four compounds came from the three types of *M. foetida* Lour. (type Limus, Batu and Manis). In PC2, it was obtained that the feature vectors were 27 compounds with 11 compounds (C14, C15, C16, C23, C24, C55, C56, C57, C64, C87, C88) which contributed the most to causing variations and were dominated by *M. sumatrana* Miq. content. A score plot that classified eight species of mangoes on the PC1 and PC2 fields represents in Figure 3. Based on the distribution of mangoes on this graph, four quadrants are formed consisting of QI (positive-positive), QII (positive-negative), QIII (negative-negative), QIV (negative-positive). In general, the PCA results show agreement with the groupings obtained from cluster analysis, even though some species that belonged to the same cluster were not located in the same quadrant.

DISCUSSION

The alkaloid compounds detected consisted of 18 compounds. Based on the results of the analysis, alkaloid compounds can be the key compounds that differentiate between species because certain alkaloid compounds are only found in certain species of mangoes. According to Reynolds (2007), metabolite compounds can be used as characters in distinguishing between species because the chemical structures formed and their biosynthetic pathways are specific and limited to the related organisms.

Table 4. Functions of the phenolic group compounds**.**

Compound Name	Derivative	Function
Quercetin	Flavonoids	Antioxidant, antibacterial, antifungal, anti-edema, anti-inflammatory, antitumor, anti-cancer, anti-ulcer, antiviral[1]
Luteolin		Uric acid[2], anticalculi[3]
Sophoricoside		Hepatoprotector[4]
Liquiritigenin		Induction activity on damage to DNA[5]
Myricetin		Anti-inflammatory and antioxidant[6]
Hyperoside		Antifungal [7]
Scortechinone F	Flavonoids (Xanthones)	Antimicrobial[8]
4-Methoxy-2-(2-methyl-2-propanyl)phenol	Phenylpropanoid	Aroma therapy [9]
4-Allyl-2-Methoxy-3-Methylphenol		
2,3',4,5'-Tetramethoxystilbene		
2,3-Dihydroxy-5-{[2-(2-methoxyethoxy)ethoxy]carbonyl} phenyl 3,4,5-trihydroxybenzoate	Gallic acid	Antibacterial, antiviral, analgesic and anti-oxidant and can act as anti-HIV and anti-carcinogenic [10]

Sources: 1. Lakhanpal and Rai (2007) 2. Owen *et al.,* (2003) 3. Dhianawaty *et al*., (2003) 4. Li and Lu (2018) 5. Arung *et al.,* (2009) 6. Narwanto *et al.,*2018 7. Li *et al.,* 2005 8. Araújo *et al.,* (2019), 9. Carvalho (2015) 10. Junaidi and Anwar (2018).

Furthermore, there were six compounds classified as alkanes.

One of the alkanes, 4,7,7-Trimethyl-3-Oxobicyclo (2.2.1) Heptane-1-Carboxylic Acid was found in all species of mangoes analyzed in this study except for *M. foetida* (type. Limus). This is presumably because of these compounds in the *M. foetida* (type Limus) amount to very little or ≤ 50% abundance. Phenolic is a class of compounds that mostly detected in this study, namely as many as 20 compounds, including 16 flavonoids, three phenylpropanoids, and one gallic acid compound. The functions of several compounds in the phenolic group are shown in Table 4.

Mangiferin is an identical compound found in the genus *Mangifera*. Based on the results of the analysis of this study, only *Mangifera* sp.2 (MH) was detected to contain mangiferin. According to Mann and Kaufman (2012), there are several factors influence whether a compound is detected or not in the test sample, which are differences in isolation methods, equipment used, plant origin, climate, plant structure, and plant age. Previous studies on metabolite diversity in several varieties of mangoes revealed that the quantity of mangiferin in the sample could be measured even in a small amount through high performance liquid chromatoraphy (HPLC) (Pradhan *et al.,* 2018). Whereas in this study, the sample was analyzed semi-quantitatively and only analyzed the dominant compound with an abundance of ≥ 50%. The selection of metabolites analyzed with a limit of 50% aims to eliminate the impact of environmental factors on the resulting phytochemical compounds. This is thought to have caused no mangiferin detection in the seven mango species apart from *Mangifera* sp.2 (MH). Mangiferin has the same structure as morin. The results of this study resulted in the morin compound found in the *Mangifera foetida* Lour. (type Batu and Manis), *Mangifera* sp.2 (MH), and *Mangifera kemanga*.

Some of the phenolic compounds found, some compounds that have an important role in the field of medicine, espesifically quercetin/morin which is antioxidant and research by Shi *et al.* (2014) shows the presence of anti-cancer activity. With the detection of these compounds, wild mangoes have the potential to be used as a medicinal ingredient. Another class of compounds

found in this study are amino acids. Amino acids are the main components of proteins, playing an important role in metabolic processes (Mandila and Hidajati, 2013; Evi *et al.,* 2017). Furthermore, in the benzene group, several compounds that are known to be used are aniline, benzoic acid, and benzamide. The benzamide can be used as an antibacterial agent (Rai and Singh, 2011; Wisnu *et al.,* 2018).

This study also obtained fatty acid compounds, namely sterols and fatty acids. Phytosterols are known as sterols in plants. Phytosterols can be used to lower cholesterol by 19-20% by binding to cholesterol in digestion (Triliana *et al.,* 2012). Fatty acyl is used as a surfactant, emulsifier, nutrient, energy source and energy reserve for human membrane stabilizers (Human metabolome database). Then the organic aromatic compound is pyrimidinamine which is commonly used as an insecticide and is widely used as a mosquito repellent (Yunis *et al.,* 2016). Another compound that acts as an insecticide is a diterpenoid derivative (Bahri, 2005).

Khelloside which is a member of the furochromone group was detected in *M. laurina*, *M. magnifica*, *M. quadrifida* and *Mangifera* sp.1 (MBS). This compound can be used as an antihyperlipidemic, lowering low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and total cholesterol (PubChem database). In general, furochromone is a class of compounds that can be used to reduce pain, renal colic and urethral spasms and facilitate the passage of urethral stones (Abu-Haashem and El-Shazly, 2015). Furthermore, the compound detected is a carboxylic acid group that plays a role in binding heavy metal ions or as a heavy metal bioadsorbent (Suhartini, 2013). In addition, there is also acetic acid which plays a role in inhibiting the growth of reduction of spore germination and outgrowth with concentration of 2% (Valenzuela-Martines *et al.,* 2010).

The results of the chromatogram data analysis also detected several compounds that could not be classified into a larger compound group due to limited information in the database. However, several publications indicate that these compounds have been tested for effectiveness. These compounds are isoxepac (6,11-Dihydro-11-oxodibenz [b.e] oxepin-2-acetic acid), this compound acts as a non-steroidal anti-inflammatory with analgesic and antipyretic activity (Lassmann, 1977). Furthermore, the compounds 3R, 4S, 4aS, 9bS) -3 - {(Cyclopentylacetyl) [2- (2-methoxyphenyl) ethyl] amino} -8-formyl-4-hydroxy-N- (2-hydroxyethyl) -6-methoxy -3,4,4a, 9b-tetrahydrodibenzo [b, d] furan-1-carboxamide is a compound that is used as an antiviral and anti-influenza (Yu *et al.,* 2017).

The variety of compounds produced by each type of mango is influenced by internal factors (genes) and external (environmental) factors. According to Mann and Kaufman (2012), a species with other species will have a very different composition in the quality and quantity of secondary metabolites produced. This is very possible because the metabolic pathways that are passed by each species are different, so that it will produce key compounds that are characteristic to distinguish it from other species (Setyorini and Yusnawan, 2016). Externally, the production of secondary metabolites is influenced by growing environmental conditions that can trigger stress in plants, so that plants make adaptation efforts in the form of increasing, decreasing or producing certain metabolite compounds which under normal conditions would not be found (Basu *et al.,* 2016).

The results of botanical exploration by Kostermans and Bompard (1993) divided the genus *Mangifera* into subgenera *Mangifera* and subgenera *Limus* (Marchand) Kosterm based on the shape of the flower disc. However, the Kosterman and Bompard grouping was not supported by the metabolites analyzed. This is because the chemical compound expressed is not related to the appearance of the disc character which is the key to

differentiating between the two subgenera. Based on the dendrogram shown in Figure 1, the sub-cluster IA is divided into IA.I and IA.II with a similarity of 74%. Subclaster IA consisting of *Mangifera foetida* (type. Batu, Limus and Manis), *Mangifera* sp.2, *M. kemanga, M. magnifica*, and *M. laurina*. The position of *Mangifera foetida* is in line with the results of PCA analysis which shows that the three types are grouped in QIII because they contain the highest levels of flavonoid compounds. *M. sumatrana* was in the IB sub-cluster with a similarity of 71.5%. PCA analysis showed position of *M. sumatrana* at QI, gather with *M. laurina*. *M. sumatrana* obtained 11 compounds from 27 compounds as feature vectors which contributed the most to causing variations and was dominated by *M. sumatrana* Miq. (Figure 2). This is what allows this type to be separated in the IB sub-cluster. Meanwhile, *Mangifera* sp.1 *and M. quadrifida* species are in the second cluster and are in QII in the PCA analysis.

The uniqueness of the position shown by QI to *M. sumatrana* Miq. which is treated as a synonym of *M. laurina* Bl. based on the classification compiled by Kostermans and Bompard (1993).This is in line with the report by Fitmawati *et al*,. (2013) that this species is closely related which sits in the same clad as *M. laurina* Bl. and *M. indica* L. reviewed based on morphological characters. However, information based on phylogenetic studies using and molecular analysis using the ITS sequence showed that *M. sumatrana* Miq. not a synonym to *M. laurina* Bl (Ariffin et al., 2015). Based on our analysis, both of them only showed a similar metabolite value of 76% and belong to a different subgroup within group I (Table 2). These two taxonomic pieces of evidences complement each other and provide confirmation of *M. sumatrana* Miq status.

In addition, several of compounds from the alkaloid, alkane, amino acids, benzene, benzoic acid, and fatty acyl groups are only owned by *M. sumatrana* Miq. Conversely, a number of certain compounds from the phenolic group (gallic acid), amino acids, benzene, and benzoic acid are also only owned by *M. laurina* Bl. So it can be reported that *M. sumatrana* Miq. not a synonym of *M. laurina* Bl and contradicts the morphological classification by Kostermans and Bompard (1993). PCA analysis shows *M. sumatrana* Miq. and *M. laurina* Bl. are in the same quadrant, namely QI. Both of them contain the compound 1- (Cyclohexylmethyl) -5'-O-tritylinosine (point C79 on the biplot), but both of them show that they are quite far away because there are certain compounds that only *M. sumatrana* Miq has. such as fatty acyl ((1S, 2S, 7R, 16S, 18S, 20R) -11-Hydroxy-20-(hydroxymethyl) -16-methoxy-6,6,7,20-tetramethyl-10,18-bis (3- methyl-2-buten-1-yl) -3,8,19-). The morphology of these two species are distinguished by the character of the fruit. The distinctive characteristics of the *Mangifera sumatrana* is large and flat, fruit break type is prominent, quantity of fibre in pulp and stone high. *Mangifera laurina* fruit is small, round in shape and fruit break type is percebtible. Geographically, the distribution of *M. sumatrana* and *M. laurina* is also different. *M. sumatrana* is found in low land areas (less than 100 mbsl), collected in Palembang, Pekanbaru, Kampar and Pariaman, while *M. laurina* was found throughout the island of Sumatra, especially in the highlands (altitude up to 2000 mbsl) (Fitmawati *et al.,* 2013). This further emphasizes that they are not the same type or synonym and the status of both needs to be reconsidered.

In this study, the isolation of certain secondary metabolites has not been carried out which can be used as specific markers to differentiate between species. Marker compounds are needed to confirm the existence of a plant extract and can also be applied to proving the authenticity of species, as well as enabling the discovery and development of new drugs (Kushwaha *et al.,* 2010). Further research is needed to reveal new facts about the position of *Mangifera* sp.2 (MH) in mango classification to ensure its

position as a new type or similar to published species.

Another interesting thing found in this study is that there are two species of rare mangoes from Sumatra, which consist of *M. quadrifida* Jack. and *Mangifera* sp.1 (MBS) is in cluster II. Based on PCA analysis, the two species tend to form separate components from other QII members. Based on the similarity of morphological characters and lines of evolution, both are also located on the same clade (Fitmawati *et al.,* 2013). Phylogenetically, *M. quadrifida* Jack. originating from Central Sumatra, it is the most basal taxon in the genus *Mangifera* and the former wild species found in Sumatra (Fitmawati *et al.,* 2016).

Recommendations that the *Mangifera* sp.1 and *Mangifera* sp.2 species as new species are compounds found in these two species that are not found in other species. *Mangifera* sp. 1 (MBS) has nine distinctive compounds that exist in this type, including 4-((2-oxohexadecanoyl)amino)butanoic acid, (4E)-1-[2-(Diethylamino)ethyl]-4-[hydroxy(4-isobutoxy-3-methylphenyl)methylene]-5-(3-phenoxyphenyl)-2,3-pyrrolidinedione, 1-[(1'-Methyl-1,4'-bipiperidin-4'-yl)methyl]-3-[2-methyl-2-(1-piperidinyl)propyl]urea, Gardenin E, (13R)-13-[(2R,3R,4R,5R)-3,4-Dihydroxy-5-(hydroxymethyl)-2-pyrrolidinyl]-13-hydroxy-5-oxotridecyl β-D-glucopyranoside, 3β)-Cholest-5-en-3-yl (15,16-dihydroxy-4,7,10,13-tetraoxahexadec-1-yl)carbamate, 2-{(E)-[6-(3,4-Dimethoxyphenyl)-8-ethoxy-1,3-dimethyl-4H-cyclohepta[c]furan-4-ylidene]amino}aniline, 2-(3,4-Dimethylphenyl)-4-[(2E)-2-{6-oxo-5-[3-(5H-tetrazol-5-yl)phenyl]-2,4-cyclohexadien-1-ylidene}hydrazino]-5-phenyl-2,4-dihydro-3H-pyrazol-3-one and Propane, 1,3-bis(4,6-diphenyl-1,3,5-triazin-2-yl). *Mangifera* sp.2 (MH) contains three compounds include Mangiferin, 2-Hexyl-3,5-Dipentylpyridine, 3R,4S,4aS,9bS)-3-{(Cyclopentylacetyl)[2-(2-methoxyphenyl)ethyl]amino}-8-formyl-4-hydroxy-N-(2-hydroxyethyl)-6-methoxy-3,4,4a,9b-tetrahydrodibenzo [b,d]furan-1-carboxamide. Among the compounds above, there are compounds that have not been detected in the ChemSpider formula database so that the names of the compound formulas have not yet emerged.

The results of this study are the first steps in chemotaxonomic research of Sumatran wild mangoes. It is hoped that the species that have the potential as new species can be justified and validated through further research. Biochemical characterization using sophisticated and more specific methods is needed to facilitate a more comprehensive taxonomic study of mangoes. Information on the certainty of taxonomic status is vital for selection and breeding, utilization, management, and conservation of wild Sumatran mangoes.

CONCLUSION

Based on the metabolic profile using LCMS obtained 2000 compound molecules and selection of 88 compounds with high abundance to minimize the impact of biased enviromental factors. Based on phytochemical information using LCMS, the recommended species *Mangifera* sp.1 and *Mangifera* sp.2 are new species. The results obtained are expected to be useful in supporting the justification and validation of the position and type of mango to support further research and conservation activities. These results indicate the position of *M. sumatrana* and *M. laurina* not as synonyms, but recommended to be different species.

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REFERENCES

Abu-Haashem AA, El-shazly M (2015). Synthesis, reactions and biological activities of furochromones: A review. *Eur. J. Med. Chem*. 90(1):633-655.

Araújo J, Fernandes C, Pinto M, Tiritan MA (2019). Chiral derivatives of xanthones with antimicrobial activity. *Molecules* 24 (2):1-29.

Ariffin Z, Md Sah MS, Idris S, Hashim N (2015). Genetic diversity of selected *Mangifera* species revealed by inter simple sequence Repeats Markers. *Int. J. Biodiver.* 2015(1):1–8.

Arung ET, Wicaksono DB, Sandra F (2009). Prenylated flavonoids as potential anti-cancer compounds. *Cermin Dunia Kedokteran (CDK)* 36(1):20–22.

Bahri S (2005). Isolated terpenoid compounds from pepper leaves (*Piper nigrum*, Linn) and their bioactivity test against Pests *Callosobruncus chinensis*. *J. Sains Tek.*11(3):158–166.

Basu S, Ramegowda V, Kumar A, Pereira A (2016). Plant adaptation to drought stress. *F1000 Res.* 5:1-10.

Borden SA, Palaty J, Termopoli V, Famiglini G, Cappiello A, Gill CG, Palma P (2020). Mass spectrometry analysis of drugs of abuse: Challenges and emerging strategies. *Mass Spectrometry Reviews* 39(5):703–744.

Carvalho AA, Andrade LN, De Sousa EBV, De Sousa DP (2015). Antitumor phenylpropanoids found in essential oils. *Bio-Med Res. Int.* 2015:1-21.

Dhianawaty D, Padmawinata K, Soediro I, Andreanus A, Soemardji (2003). Isolation, characterization, and preventive anticalculi activity test of luteolin 7- O -glucoside from *Sonchus arvensis* L. leaves with matrix-glycolyc acid method on rats. *J. Bionatura* 5(3):196-202.

Eiadthong W, Nakatsubo F, Yonemori K, Sugiura A (2000). Chemotaxonomic studies on some *Mangifera* species by chemical compositions in the bark. *Acta Hort.* 509:143-152.

Esslinger S, Riedl J, Fauhl-Hassek C (2014). Potential and limitations of non-targeted fingerprinting for authentication of food in official control. *Food Res. Int.* 60:189–204.

Evi S, Maya, Mala N, Abdullah A (2017). Amino acid profile and bioactive compounds of Sea Horse *Hippocampus comes*. *J. Ilmu dan Teknol. Kelaut. Trop*. 9(2):605–618.

Farag MA, Porzel A Wessjohann LA (2012). Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques. *Phytochemistry* 76:60–72.

Fitmawati F, Resida E, Kholifah SN, Roza RM, Almurdani M, Emrizal E (2020). Phytochemical screening and antioxidant profiling of Sumatran wild mangoes (*Mangifera* spp.): a potential source for medicine antide generative effects. *F1000 Res.* 9(220):1-13.

Fitmawati, Hayati I, Sofiyanti N (2016). Using ITS as a molecular marker for *Mangifera* species identification in Central Sumatra. *Biodiversitas*17(2):653–656.

Fitmawati, Swita A, Sofyanti N, Herman (2013). morphological relationship analysis of *Mangifera* from Central Sumatra. *Floribunda* 4(7):169–174.

Ganneru S, Shaik H, Peddi K, Mudiam MKR (2019). Evaluating the metabolic perturbations in *Mangifera indica* (mango) ripened with various ripening agents/practices through gas chromatography - mass spectrometry based metabolomics. *J. Sep. Sci*. 42(19): 3086–3094.

Hidayat T, Pancoro A, Kusumawaty D, Eiadthong W (2012). Development matK gene as DNA barcode to assess evolutionary relationship of important tropical forest tree genus *Mangifera* (Anacardiaceae) in Indonesia and Thailand. *J. Teknol.* 59(1):17-20.

Hiralben DV, MK Mandavia, Radadiya N,JK Jadav, BA Golakiya (2018). Metabolite profiling of mango cv. Kesar leaf using gas chromatography-mass spectrometry. *Int. J. Chem. Stud.* 6(4):2731–2735.

Jain C, Khatana S, Vijayvergia R (2019). Bioactivity of secondary metabolites of various plants: A review. *Int. J. Pharm. Sci. Res.* 10(2):494–504.

Junaidi E, Anwar YAS (2018). Antibacterial and antioxidant activity of gallic acid of local fruit skins produced with Tanase. *ALCHEMY J. Penelit. Kim.* 14(1):131-142.

Khairan, Jenie UA, Sudibyo RS (2009). Fragmentation studies of Δ6, 7-anhidroeritromisin-A by liquid chromatography-mass spectroscopy (LC-MS). *Indo. J. Chem.* 9(3):491–499.

Kostermans AJGH, Bompard JM (1993). *The Mangoes: Their Botany, Nomenclature, Horticulture and Utilization*. London (UK). Academic Press.

Kushwaha SKS, Kushwaha N, Maurya N, Rai AK (2010). Role of markers in the standardization of herbal drugs : A Review. *Arch. Appl. Sci. Res.* 2(1):225–229.

Lakhanpal P, Rai DK (2007). Quercetin: A versatile flavonoid. *Int. J. Med. Update* 2(2):22-37.

Lassmann H, Jurecka W, Lassmann G , Gebhart W, Matras H, Watzek G (1977). Different types of benign nerve sheath tumors light microscopy, electron microscopy and autoradiography. Virchows Arch. *A Path. Anat. and Histol*. 375:I97-210.

Li S, Zhang Z, Cain A, Wang B, Long M, Taylor J (2005). Antifungal activity of camptothecin, trifolin, and hyperoside isolated from *Camptotheca acuminata. J. Agric. Food Chem.* 53(1):32−37.

Li W, Lu Y (2018). Hepatoprotective effects of sophoricoside against fructose-induced liver injury via regulating lipid metabolism, oxidation, and inflammation in mice. *J. food Sci.* 83(2):552-558.

Luo F, Lv Q, Zhao Y, Hu G, Huang G, Zhang J, Sun C, Li X, Chen K (2012). Quantification and purification of mangiferin from Chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H_2O_2-induced stress. *Int. J. Mol. Sci.* 13(9):11260-11274.

Mandila SP, Hidajati N (2013). Identification of Amino Acids From Silk Worms (*Tubifex* sp.) Extracted Using Acetic Acid And Lactic Acid Solvents. *UNESA J. Chem*. 2(1):103–108.

Mann RS , Kaufman PE (2012). Natural product pesticides: their development, delivery and use against insect vectors. *Mini-Rev. in Organic Chem.* 9(2):185-202.

Narwanto MI, Rahayu M, Soeharto S, Widodo MA (2018). Identification and in silico analysis of anti inflammation and anti oxidant potentials of polyphenol compounds in methanol extract of *Tamarindus indica* seeds. *J. Agromed. Med. Sci.* 4(1):13–17.

Nurmaida N, Darusman LK, Rafi M, Heryanto R (2018). metabolite profiling of tabat barito (*Ficus deltoidea*) using UPLC-QTOF-MS/MS. *J. Pure App. Chem. Res*. 7(2):100-108.

Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem. Toxicol.* 41(5):703-717.

Pardo-Andreu GL, Paim BA, Castilho RF, Velho JA, Delgado R, Vercesi AE, Oliveira HCF (2008). *Mangifera indica* L. extract (Vimang®) and its main polyphenol mangiferin prevent mitochondrial oxidative stress in atherosclerosis-prone hypercholesterolemic mouse. *Pharmacological Research* 57(5):332–338.

Pham-Huy LA, He H, Pham-Huy C (2008). Free radicals, antioxidants in disease and health lien. *Int. J. Biomed. Sci.* 4(2):89-96.

Pradhan ID, Dutta M, ChoudhuryK, De B (2018). Metabolic diversity and in vitro pancreatic lipase inhibition activity of some varieties of *Mangifera indica* L. fruits. *Int. J. Food Prop.* 20(3):3212–3223.

Rai D, Singh RK (2011). Synthesis and antibacterial activity of benzamides and sulfonamide derived from 2- amino-5-bromo/nitropyridine against bacterial strains isolated from clinical patients. *Indian J. Chem.* 50(7):931–936.

Reynolds T (2007). The evolution of chemosystematics. *Phytochem.* 68:2887–2895.

Ronchi SN, Brasil GA, do Nascimento AM, de Lima EM, Scherer R, Costa HB, Romão W, Boëchat GAP, Lenz D, Fronza M, Bissoli NS, Endringer DC, de Andrade TU (2015). Phytochemical and in vitro and in vivo biological investigation on the antihypertensive activity of mango leaves (*Mangifera indica* L.). *Ther. Adv. Cardiovasc. Dis.*9(5):244–256.

Sartono B, Affendi FM, Syafitri UD, Sumertajaya IM, Anggraeni Y (2003). *Multiple variable analysis*. Bogor. IPB Press.

Schulze-Kaysers N, Feuereisen MM, Schieber (2015). Phenolic compounds in edible species of the Anacardiaceae family – A review. *RSC Adv.* 5(89):73301–73314.

Setyorini SD, Yusnawan E (2016). Increased secondary metabolite content of various peanut plants as biotic stress response. *Iptek Tanaman Pangan* 11(2):167-174.

Shi ZH, Li NG, Tang YP, Shi QP, Zhang W, Zhang PX, Dong ZX, Li W, Zhang X, Fu HA, Duan JA (2014). Synthesis, biological evaluation and SAR analysis of O-alkylated analogues of quercetin for anti-cancer. *Bioorg. Med. Chem. Lett.* 24(18):4424-4427.

Shyur LF, Yang NS (2008). Metabolomics for phytomedicine research and drug development. *Curr. Opin. Chem. Biol.*12(1):66–71.

Suhartini M (2013). Modification of banana peel waste for Mn (II) AND Cr (VI) metal ion adsorbents. *Pus. Apl. Teknol. Isot. dan Radiasi – BATAN* 14(2):229–234.

Suparman S, Pancoro A, Hidayat T (2013). Phylogenetic analysis of *Mangifera* base on rbcl sequences, chloroplast DNA. *Scientific Papers. Series B. Horticulture* 57:235-240.

Tank J, UkaleDU, MapareV, Dhamangaonkar B, Cukkemane N, Cukkemane AA (2016). Metabolic profiling of wastes from raw and ripe mango seeds to comprehend its beneficial medicinal and cosmetic properties for commercialization. *J. Agric. Stud.* 5(1):1-14.

Taupik M, Djuwarno EN , Mustapa MA, Sahumena MH (2020). Identification and Study of Phenylbutazone Confirmed Herbal Fragmentation Patterns Using Liquid Chromatography Mass Spectormetry (Lcms). *Scientia J. Far. Kes.* 10(2):243-251.

Triliana R, Soeatmadji DW, Kalim H (2012). Effect of phytosterol supplementation therapy on plasma fat profile, apolipoprotein (apo) b-48 levels, and aortic foam cell count in post-radiated atherogenic mice. *J. Exp. Life Sci.* 2(22):70–81.

Valenzuela-Martinez C, Pena-Ramos A, Juneja VK, Korasapati NR, BURSON DE,, Thipparedddi H. (2012). Inhibition of *Clostridium perfringens* Spore Germination and Outgrowth by Buffered Vinegar and Lemon Juice Concentrate during Chilling of Ground Turkey Roast Containing Minimal Ingredients. *J. Food Prot.* 73(3):470–476.

Wauthoz N, Balde A, Balde E, Van Damme M, Duez P (2007). Ethnopharmacology of *Mangifera indica* l. bark and pharmacological studies of its main c-gucosylxanthone, mangiferin. *Int. J. Biomed. Pharm. Sci.* 1(2):112–119.

White IR, Blake RS, Taylor AJ, Monks PS (2016). Metabolite profiling of the ripening of Mangoes *Mangifera indica* L. cv. 'Tommy Atkins' by real-time measurement of volatile organic compounds. *Metabolomics* 12(3):1–11.

Wisnu EDOU, Sary IP Pratoko DK (2018). Synthesis and antibacterial activity assay of n-phenyl-4-chlorobenzamide. *E-J.Pustaka Kesehata*n 6(1):212–217.

Yonemori K, Honsho C, Kanzaki S, Eiadthong W, Sugiura A (2002). Phylogenetic relationships of *Mangifera* species revealed by ITS sequences of nuclear ribosomal DNA and a possibility of their hybrid origin. *Plant Syst. Evol.* 231(1):59-75.

Yu Y, Zheng J, Cao L, Li S, Li X, Zhou HB, Liu X, Wu S, Dong C (2017). Furan-carboxamide derivatives as novel inhibitors of lethal H5N1 influenza A viruses. *RSC Adv.* 7(16):9620–9627.

Yunis T, Wahyono M, Mw O (2016). Use of mosquito's insecticide and dengue prevention activities at DKI Jakarta and Depok. *J. Epidemiol. Kesehatan Indonesia* 1(1):35–40.